

are deeply inserted in the membrane and contact the methyl termini of the lipid acyl chains. This antiparallel distribution is also observed for a much larger segment of gp41 that includes the fusion peptide. A very different registry distribution is detected for the non-functional V2E mutant and suggests that the amino acid content of the antiparallel sheet is correlated to membrane fusion.

### 3218-Symp

#### Membrane Dynamics and Lipid Interactions of Influenza Fusion Proteins Peter Kasson.

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Viral fusion peptides interact with lipid membranes to promote membrane fusion and viral entry. Data on fusion-defective mutants of influenza virus suggest that these interactions are in fact required for efficient fusion. Fusion peptides appear to be at least structurally plastic if not highly dynamic, adopting multiple conformations and potentially multiple interaction modes with lipid membranes. It remains unclear, however, how these structural properties are linked to physiologic function. We have used molecular dynamics simulation to probe the conformational transitions and membrane interactions of influenza hemagglutinin. Based on these simulations, we obtain models of 1) how fusion peptides can lower the activation barrier to the first lipidic intermediates in membrane fusion, 2) how structural plasticity may be important to this activity, and 3) how inactive mutants of influenza fusion peptides may be functionally impaired. We have also performed functional experiments on how the membrane environment affects fusion kinetics of live influenza virus. Correlation of models from simulation with spectroscopic and functional data can help to further elucidate the mechanisms by which fusion peptides promote membrane fusion and how structure and dynamics relate to function.

### 3219-Symp

#### How SNARE Assembly and Folding may Drive Membrane Fusion Lukas K. Tamm.

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Soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-mediated membrane fusion is driven by the assembly of the four-helix bundle of the SNARE complex. Three SNARE proteins form the SNARE complex that is responsible for rapid exocytosis of synaptic vesicles at presynaptic membranes of neurons: synaptobrevin (or VAMP) 2 is the single vesicle SNARE and syntaxin 1A together with synaptosomal-associated protein 25 (SNAP-25) forms the acceptor SNARE complex on the presynaptic plasma membrane. While synaptobrevin and syntaxin are single helix-spanning integral membrane proteins, SNAP-25 is a palmitoylated soluble protein. We have solved the monomeric prefusion structures of membrane-anchored synaptobrevin and syntaxin by solution NMR methods and studied their membrane association with site-directed spin-label EPR spectroscopy. Contrary to the common notion that the soluble portions of SNAREs are completely disordered prior to assembly, we found that certain regions have higher propensities to pre-fold into helical segments and exchange with the membrane surface than other regions. Supported by fluorescence interferometric measurements in supported membranes, we propose a mechanism of SNARE assembly that could provide the energy to drive tight membrane docking (by formation of a partially folded trans-SNARE complex) to membrane fusion (by formation of the completely folded cis-SNARE complex).

### 3220-Symp

#### Structural Rearrangement of the Ebola Virus VP40 Protein Begets Multiple Functions in the Virus Life Cycle

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Structural basis for ebolavirus matrix assembly and budding; protein plasticity allows multiple functions. Proteins, particularly viral proteins, can be multifunctional, but the mechanism(s) behind this trait are not fully understood. Here, we illustrate through multiple crystal structures, biochemistry and cellular microscopy that a protein of the Ebola virus, termed VP40, rearranges into different structures, each with a distinct function required for the ebolavirus life cycle. A butterfly-shaped VP40 dimer trafficks to the cellular membrane. There, electrostatic interactions trigger rearrangement of the polypeptide into a linear hexamer. These hexamers construct a multi-layered, filamentous structure that is critical for assembly and budding of nascent virions and resembles tomograms of authentic virions. A third structure of VP40, formed by a different rearrange-

ment pathway, is not involved in virus assembly, but instead exists only in infected cells, where it uniquely binds RNA to regulate viral transcription. These results provide a functional model for Ebola virus matrix assembly and the other roles of VP40 in the virus life cycle, and demonstrate how a single, wild-type, unmodified polypeptide can assemble into different structures for different functions.

## Symposium: Biophysics of Cell Division and Spatial Relationships

### 3221-Symp

#### Direct Evidence for Sister Kinetochores Fusion in Meiosis I

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For decades, biologists have wondered how replicated sister chromatids, which always separate from one another during normal mitotic cell division, instead co-migrate together during phase I of meiosis, the specialized division that produces eggs and sperm. Genetic, cytological, and biochemical studies using budding yeast have suggested that sister kinetochores, which link the sister chromatids to microtubules and drive their movement, may be mechanically fused by a meiosis I-specific kinetochore-binding factor called monopolin. But thus far the evidence for monopolin-dependent sister kinetochore fusion has been indirect, and the possibility that monopolin could instead promote co-migration by binding to and inhibiting one of the two sister kinetochores has not been excluded. To test these ideas directly, we are isolating native meiotic and mitotic kinetochore particles, reconstituting their function in vitro, and applying advanced tools for manipulating and tracking individual molecules. Using laser trapping, we find that kinetochore particles from meiosis I form substantially stronger attachments to dynamic microtubule tips than those from mitosis or from meiosis II. The high strength of meiosis I particles is lost if DNA replication is blocked during pre-meiotic S-phase, demonstrating that sisters are required. High strength is also lost if monopolin is disrupted prior to kinetochore particle isolation and, conversely, the strength of mitotic kinetochores can be increased by artificially inducing monopolin expression. Quantitative fluorescence microscopy also confirms that meiosis I particles have more copies of the core microtubule-binding component, Nuf2. Together these data suggest that sister kinetochores are mechanically fused by the monopolin complex during meiosis I, in such a way that the microtubule-binding elements from the two sister kinetochores can cooperate together to form a single attachment site.

### 3222-Symp

#### Intrinsic and Extrinsic Noise in the Flagellar Length Control System Wallace Marshall.

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The control of organelle size is currently an open question in cell biology. The eukaryotic cilium or flagellum provides a simple, one-dimensional model organelle in which to study the mechanisms of size control. We have previously analyzed the dynamics of transport within the flagellum and found evidence for a control system regulating transport as a function of length, coupled with an avalanche-like behavior that generates power-law distributions of transport mediator sizes. Here, we analyze fluctuations in flagellar length as a new way to probe the mechanisms of size control, and link these observations with our previous results using a simple noise model. A similar approach can be applied to probe size control mechanisms in any organelle.

### 3223-Symp

#### Cytoskeletal Assembly Under Confinement Daniel Fletcher.

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How the crowded confines of a cell influence the assembly and organization of intracellular structures remains a mystery. While some cytoskeletal structures have been successfully formed in vitro from purified protein components, biochemical reconstitution outside the complex environment of the cell carries the inevitable risk of removing too much context and therefore sacrificing important physical constraints that guide the system's behavior. One such constraint is the size of cells. This talk will describe recent work investigating the effect of volumetric confinement on the assembly of mitotic spindles in *Xenopus* egg extract, as well as experimental methods for recreating physical constraints for in vitro reconstitutions. This work contributes to the growing